MELANOGENESIS IN HUMAN SKIN FOLLOWING EXPOSURE TO LONG-WAVE ULTRAVIOLET AND VISIBLE LIGHT*

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Most speculative thinking about the effects of solar radiation on the formation, migration and darkening of pigment in human skin centers about experimental data, some of which have been substantiated by several investigators and some of which have been accepted without detailed verification. The purpose of this article is to attempt to probe thoroughly the phenomenon of the "pigment-darkening" induced by light of wavelengths greater than 320 mμ. The familiar pigmentation which follows exposure to solar radiation or to ultraviolet light from artificial sources is known to involve two distinct photobiological processes. The first, generally called "*primary melanization*", consists of an erythemal response ("sunburn") followed by the formation of new pigment (melanogenesis) and the migration of melanin granules; it is initiated by wavelengths shorter than 320 mμ (*i.e.*, the so-called erythemal spectrum) with maximal efficiency at 297 and 254 mμ. The second, which has been referred to as "*immediate pigment-darkening*" (IPD), is evoked by wavelengths greater than those which induce primary melanization. The two processes differ in several ways. In contradistinction to primary melanization, pigment-darkening is known to begin immediately after exposure to light, without any latent period; it appears to be an oxygen-dependent, photochemical reaction in which preformed, colorless or lightly colored melanin is oxidized to the dark form. The wavelengths which initiate this reaction have been reported to lie in the range between 300 and 420 mμ with a broad maximum near 340 mμ (Fig. 1) (1, 2).

Darkening of the pigment of normal skin immediately after irradiation with long-wave ultraviolet light was first reported by Hausser in 1938 (1). In addition to the characteristic erythema and pigmentation evoked by the sunburn spectrum, this author described erythema and pigmentation elicited by wavelengths between 300 and 700 mμ. She noticed an immediate, strong erythema which was induced by light of wavelengths between 330 and 420 mμ (maximum effect at 385 mμ). Hausser reported that following exposure to ultraviolet of 385 mμ for between 20 and 120 minutes, erythema appeared promptly and persisted for more than 12 hours; and that 48 hours after exposure, the erythema was superseded by pigmentation. At the end of 5 weeks, the brown pigment thus elicited appeared to have retained its maximum intensity.

Henschke and Schulze (2) studied this pigment-darkening phenomenon in more detail. They showed that exposure to ultraviolet radiation between 3,000 and 4,000 Å caused darkening of the skin which began immediately after exposure without any latent period. Microscopically, the areas of darkening showed no evidence of erythema or cellular injury; clinically, however, the erythema which began immediately after irradiation persisted for about 2 hours. Henschke and Schulze distinguished this erythemal response from pigmentation by pressing a glass slide on the skin to eliminate the redness of blood in the dilated capillaries. They determined the range of the action spectrum which caused pigment-darkening (Fig. 1) and stated that the darkening resulted from changes in melanin pigment which was present in the epidermis before irradiation. Darkening did not take place after reduction of the local blood supply of intact skin or after exposure of cadaver skin.

In an often quoted study of immediate pigment-darkening, Miescher and Minder (3) enumerated three previously described phenomena, namely: (*a*) the darkening of pigmented, living skin after long-wave ultraviolet irradiation; (*b*) the Lignac phenomenon in which excised, pigmented skin darkens when irradiated with light from a quartz lamp (4); and (*c*) the Meirowsky phenomenon in which the warming of excised, pigmented skin to 37 or 56° C, or its incubation at

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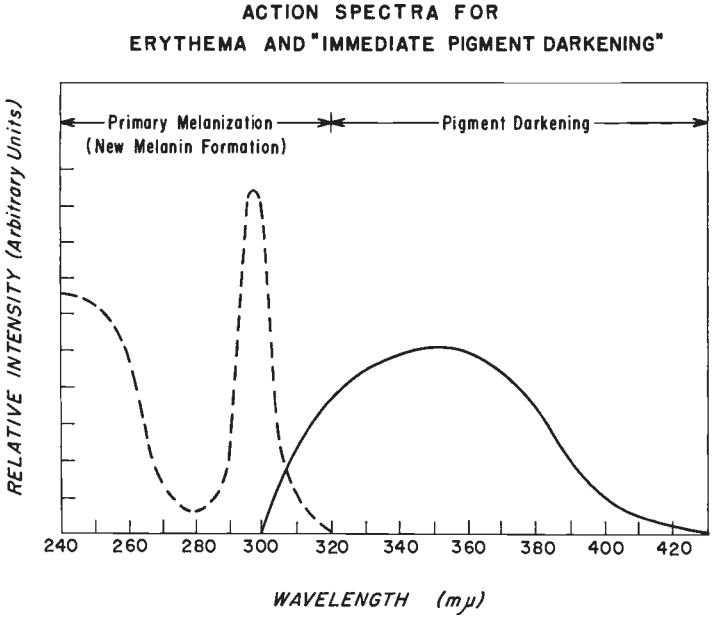


FIGURE 1

these temperatures, for 1–3 days causes darkening (5). They pointed out that these three phenomena are basically the same and that the darkening results from "oxygen-uptake by pigment already present in the epidermis." They explained that melanin is a high-molecular substance with a redox potential which exists either in a reduced form which can reduce silver, or in an oxidized form which cannot reduce silver. They thought that the melanin formed by the action of dopa-oxidase is gradually reduced when it is exposed to the redox potentials of the cell. Miescher and Minder believed that since the production of new pigment was not involved in their *in vitro* experiments, oxygen influenced the size and color of the melanin particles present in the skin, and that pigment-darkening resulted from reversion of lightly colored reduced pigment to a dark form as the consequence of oxygen consumption. It is interesting to note that of the 50 subjects studied by these authors, 39 showed immediate darkening to Grades 3 and 4. Sixteen subjects manifested no signs of inflammation, but in 23 subjects, erythema and inflammation were associated with immediate darkening. The reddening appeared without a latent period and vanished either immediately or after several days. In 11 instances, inflammation was observed alone without immediate pigment-darkening. Sharlit (6) challenged the correctness of Miescher and Minder's explanation, despite his confirmation of the pigment-darkening effect. He asserted that the Meirowsky phenomenon can be produced without atmospheric oxygen. In an oxygen-free atmosphere he observed an increase in the epidermal pigmentation of excised skin which he attributed to the production of melanin *de novo* in the cells of the epidermis and to enlargement of all previously existing melanin particles by saturation with oxygen. He postulated that the cytochrome system of the skin provides the oxygen required for this melanization. Felsher, Rubin and Rothman (7) have investigated the action spectrum of the pigment-darkening of freckles and found that it is very similar to that reported by Henschke and Schulze (Fig. 1). Bachem (8) has studied the relative time of appearance of erythema and pigmentation after irradiation by ultraviolet light of different wavelengths. He has shown that reactions to long-wave ultraviolet appear without latency, and that the pigmentation response reaches its maximum within an hour or even within a few minutes. In his study, the duration of pigment-darkening varied considerably; in some cases, the dark pigment disappeared within an hour and in others it persisted for more than a year. Recently, Rottier (9) has reported visible reactions of human skin to ir-

radiation with long-wave ultraviolet and visible light (wavelengths 366 mμ and 405-436 mμ, respectively). In addition to an erythemal response, he observed a "blue-greyish hyperemia" which appeared immediately after irradiation. No immediate pigment-darkening was observed.

In the investigation to be presented here, the effect of long-wave ultraviolet and visible light on human skin has been studied in an effort to evaluate these findings and to obtain better understanding of the phenomenon of immediate pigment-darkening (IPD). Evidence has been obtained to show that long-wave, ultraviolet and visible light, in addition to evoking immediate pigment-darkening, can initiate *melanin formation.*

METHODS AND MATERIALS

Experimental Subjects

The selection of subjects is of great importance, because immediate pigment-darkening (IPD) cannot be observed with equal ease in all types of skin. It is best seen in skin which is normally pigmented. The 21 subjects included in this experimental group included 14 fair-skinned Caucasians and 7 individuals with pigmented skin (Orientals, East Indians, and lightly pigmented Negroes).

Light Source

The monochromatic light to which the skin was exposed came from a high-intensity, ultraviolet, visible and infrared light monochromator designed and constructed in this laboratory. This monochromator† is a f/0.2 instrument in which two 5 x 5-inch diffraction gratings disperse the incident light rays emitted by a German, Osram, 2,000 watt, air-cooled, xenon arc lamp. At the site of irradiation at the 6 mm slit, the light band is approximately 75–100 Å wide. Energy output, as measured by a calibrated thermocouple, is approximately 0.1 watt/cm² at 300 mμ (first order ultraviolet) and rises to 1.0 watt/cm² at 450 mμ. The thermal output of the lamp was dispersed so that its effect on melanin formation was negligible.

Action Spectrum of Immediate Pigment-Darkening (IPD)

The range of wavelengths that effectively elicit the IPD response on the inner aspect of the forearm was determined in each of 6 subjects with pigmented skin. The amount of energy delivered to each site of irradiation (6 mm diam) during each exposure was approximately 45.9×10^7 ergs. This is equivalent to the amount of energy received during about 37 minutes exposure to the total radiation from the sun ($\lambda = 0.29$ to $\alpha\mu$) or about 78 minutes exposure to solar radiation of wavelengths between 290 and 700 mμ. According to Moon (10), at sea level with an air mass of 2, solar irradiation between wavelengths 0.29 and 1.9 μ and above equals 739.8 watts m^{-2}; irradiation between wavelengths 290 and 700 mμ equals 347.6 watts m^{-2}. In the subjects here studied, the skin was irradiated at intervals of 200 Å between 3,200 and 7,000 Å. The reaction to each exposure was graded by three observers on a visual scale of 0, +1, +2, +3, and +4. Degree of response was recorded immediately following irradiation, every 5 minutes thereafter for 30 minutes, and 1, 2, 18, 24, 48 and 72 hours and 7, 10, 30 and 60 days after the end of exposure. Within 10 minutes after irradiation, the pigment-darkening response to different wavelengths was also evaluated quantitatively on a reflectance spectrophotometer (Bausch and Lomb, Spectronic #505) which had been specially modified for this purpose.

Formation of New Melanin with Wavelengths Greater than 320 mμ

The inner aspect of the forearm of 14 fair-skinned subjects and 7 subjects with pigmented skin was irradiated in several areas with amounts of light energy ranging from 45.9 to 150×10^7 ergs at 3,600, 4,000 and 4,400 Å. Four-millimeter punch-biopsy specimens were subsequently taken from 5 of the fair-skinned subjects: from 3 subjects, prior to irradiation and 24, 48, 72, 96 and 120 or 144 hours after irradiation; from the others, prior to and 120 hours after irradiation. The tyrosinase activity of the biopsy tissue was determined by the method described by Fitzpatrick *et al.* (11). Each specimen was fixed in 2.5% formalin for 1 hour at 4° C and then divided into two parts: one half, the control, was incubated at 4–5° C in 1×10^{-1} M phosphate buffer (pH 6.8), while the other was incubated at the same temperature in a 5×10^{-3} M solution of tyrosine buffered to pH 6.8. No dopa (3,4-dihydroxyphenyl-L-alanine) was added to either control or reaction vessels. At the end of 24 hours, these specimens were placed in fresh solutions and reincubated for 24 hours at 36° C. They were then fixed in Bouin's solution, dehydrated, embedded in paraffin, cut into sections 7 and 15 mμ thick, and counterstained with hematoxylin and eosin or paracarmine.

RESULTS

Immediate Pigment-Darkening (IPD)

In this study, normally pigmented skin (Oriental, East Indian, Negro) has consistently re-

† The monochromator used was built with funds generously provided by Dr. and Mrs. John Alden of San Francisco, California. Dr. W. L. Curwen, the Department of Dermatology of the Harvard Medical School and Dr. I. A. Magnus, St. John's Hospital for Diseases of the Skin, London, helped to design and construct the monochromator.

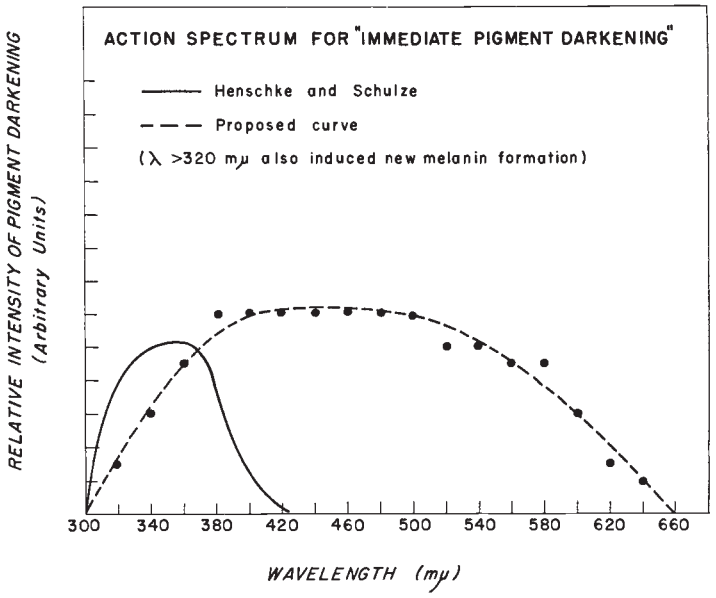


FIGURE 2

sponded to irradiation with IPD. The response seems to reach its maximal intensity immediately after the end of irradiation and thereafter gradually to diminish. Fading becomes obvious within 10-30 minutes. At a site in which the IPD was of grade 3 or 4 after exposure to 45.9 $\times$ 10^7 ergs, the darkening faded so that it was barely visible at the end of one hour and could no longer be detected 2–3 hours after irradiation. The intensity of IPD and its rate of fading seem to be dependent upon (*a*) the *normal intensity of skin pigmentation*; (*b*) the *total amount of energy* delivered to the skin during irradiation; and (*c*) the *duration* of irradiation. Fair skin, by contrast, has not shown the IPD response to irradiation with this degree of consistency. During the early parts of this study, fair skin was found for the most part to manifest the "blue-greyish hyperemia" without immediate pigment-darkening described by Rottier in 1960 (9). As work progressed, however, it became obvious that IPD can be elicited even in the lightly pigmented skin of fair subjects.

Between 48 and 72 hours after irradiation, the exposed areas again became discernible as the result of repigmentation. This repigmentation seems to be caused by newly formed melanin, because it has persisted for more than 3 months in the subjects studied. To facilitate discussion, this repigmentation which follows fading of the IPD response will hereafter be referred to as "formation of new melanin" (FNM).

IPD and FNM were observed following a 45.9 $\times$ 10^7 erg dose of ultraviolet irradiation, an amount insufficient to evoke visible erythemal response. In these experiments, this dose has been found to be between one-quarter and one-half of the amount needed to evoke a minimal erythemal response in fair skin. A visible erythemal response can be elicited in conjunction with IPD and FNM by increasing the dose of radiation. IPD alone can be produced by a 22.9 $\times$ 10^7 erg dose.

The action spectrum for IPD is shown in Fig. 2, where the dotted line represents the results obtained in this laboratory and the solid line represents the action spectrum delineated by Henschke and Schulze (2) which has been quoted and confirmed by various investigators (7, 8, 12). Rothman *et al.* (7) commented that data concerning the pigment-darkening of freckles seem "to indicate that the action spectrum extends far beyond 4,300 Å". The validity of this inference obviously could not be determined without a source of high-intensity, monochromatic radiation which could deliver a calculated amount of energy in a short period of time. With the instrument used in these experiments, it has been possible to show that the reasoning of these authors

was correct and that the action spectrum extends to 6,400 Å, with maximum effect between 4,200 and 4,600 Å. More recently, it has been found that wavelengths up to 700 mμ can evoke visible IPD if sufficient energy (in the order of 150×10^7 ergs) is delivered at the site of irradiation.

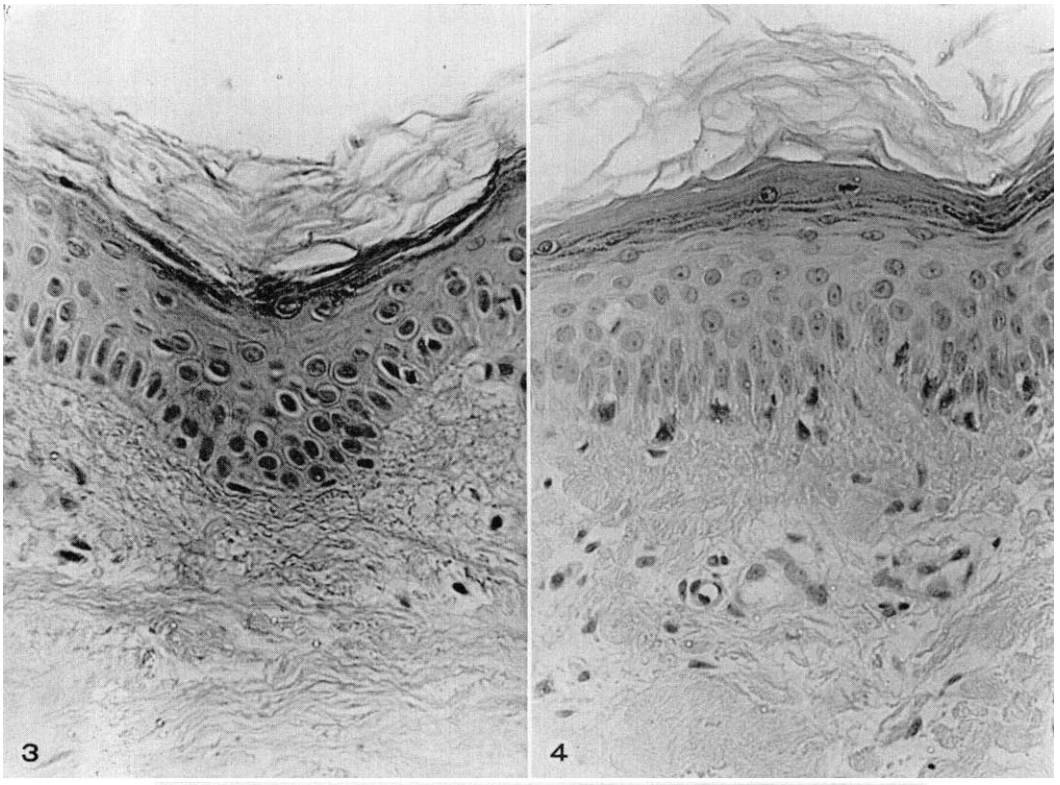
The various investigators mentioned above have explained IPD as the result of oxidation and darkening of preformed, light (leuco-) melanin, and have demonstrated that it can be inhibited in a localized area by reducing the oxygen tension of that area during exposure, *e.g.*, by pressing the skin with a glass slide, retarding circulation with a tourniquet, etc. In order to learn more about the mechanism of this reaction, an attempt was made to determine at what level within the epidermis IPD occurs. Stripping the stratum corneum 22–27 times with pressure-sensitive, cellophane tape failed to prevent IPD. Examination of the stripped, irradiated areas with a dermatoscope revealed accentuation of the pigmentary pattern of the rete ridges. The reaction must, therefore, occur in the deep, not in the superficial, layers of the epidermis. Raising the circulatory pressure of the arm to 240 mm Hg with a tourniquet or pressing the area of irradiation with a glass slide inhibited IPD. Intradermal injection of 0.1 ml adrenaline (10^{-4} M) likewise prevented the reaction locally, but neither 0.1 ml of ascorbic acid (10^{-2} M) or 0.1 ml of acetylcholine (10^{-4} M) administered intradermally exerted this inhibitory effect. Although these observations do not elucidate the nature or indicate the origin of the melanin involved in IPD, they do confirm results previously reported in the literature. It would seem that IPD is an oxidation reaction which occurs in the deeper layers of the epidermis, possibly in the basal cell layer.

Formation of New Melanin (FNM) Induced by Long-Wave Ultraviolet Radiation

The fading or disappearance of IPD 1–3 hours after irradiation with wavelengths greater than 320 mμ (360, 400, 440, 480, 520 mμ) and the reappearance of pigmentation at the same site 48–72 hours later suggest that in addition to the immediate darkening of pre-existing pigment, light of these wavelengths, and hence the energy associated with such light, is able to induce the formation of new melanin. These findings are not compatible with the concepts (*a*) that new melanin can be formed only after irradiation with ultraviolet light belonging to the so-called erythemal spectrum (*i.e.*, with wavelengths less than 320 mμ, with peaks of effectiveness at 250 and 300 mμ) (12) and (*b*) that pigment-darkening without the formation of new melanin can be induced only by light of wavelengths greater than 300 mμ (12).

These initial observations led to investigation of the relation between "erythemal spectrum" and induction of FNM by long-wave ultraviolet light. In 14 fair-skinned and 7 dark-skinned subjects, test areas were irradiated with long-wave, ultraviolet light (360, 400, 440, 460 mμ) for periods ranging from 15–25 minutes. The energy output of the monochromator at 360 mμ was 12.73×10^5 ergs/0.283 $cm^2 \cdot sec^{-1}$. Throughout this study, either second or first order ultraviolet and visible light was used for a majority of the tests with wavelengths between 340 and 600 mμ. All wavelengths shorter than 320 mμ were completely removed by Corning filters (*e.g.*, Nos. 7–59 and 3–73). Whatever heat might have developed was offset by a continuous blast of cool air. No experimental subject felt any emanation of heat. IPD developed in only 7 of the fair-skinned subjects, but a mild erythemal response could be detected visually in all 14 of these individuals immediately after irradiation. With the reflectance spectrophotometer (Bausch and Lomb, Spectronic #505) it was possible to show augmented absorption bands at 415, 542 and 575 mμ. In all fair-skinned subjects, the immediate erythemal response disappeared within 1–2 hours and reappeared after an interval of 10–18 hours. It was not due to infrared radiation or heat. In all dark-skinned subjects, there was both immediate vasodilatation (erythemal response) and IPD. In these individuals, erythema was not grossly visible 12–18 hours after irradiation, but it could be detected after this interval on the reflectance spectrophotometer. Newly formed melanin could be seen in all 21 subjects 48–72 hours after exposure if a glass slide was pressed on the irradiated area to eliminate whatever erythemal component might be present. The irradiated areas of these subjects remained pigmented for more than 60 days.

To prove that the second darkening of human skin after irradiation is due to the formation of new melanin in both fair-skinned and dark-skinned individuals, the tyrosinase activity of biopsy tissue from 5 fair-skinned subjects was



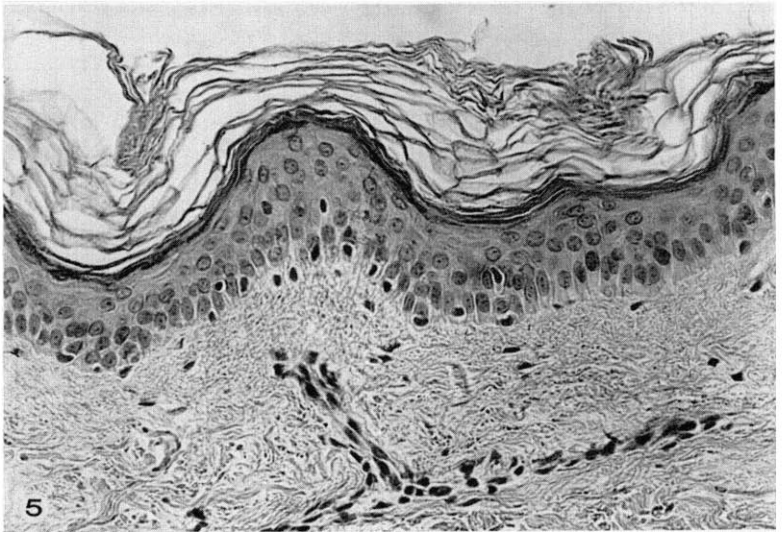


FIG. 3. Control biopsy specimen from unirradiated area after incubation in tyrosine-phosphate buffer. No dendritic melanocytes containing tyrosinase activity were observed. Specimen from irradiated area of the same subject is shown in Fig. 4. Counterstained with hematoxylin and eosin. Magnification, × 293.

FIG. 4. New melanin formation initiated by long-wave ultraviolet and visible light. Pigmented, dendritic melanocytes in the skin of a *"white"* human subject after *in vivo* exposure to wavelengths of 400 mμ and subsequent *in vitro* incubation in tyrosine-phosphate buffer. Biopsy specimen obtained 96 hours after irradiation. Counterstained with hematoxylin and eosin. Magnification, × 293. Control biopsy from the same subject is shown in Fig. 3.

FIG. 5. New melanin formation initiated by long-wave ultraviolet light (λ 360 mμ). Pigmented melanocytes in the skin of a "white" human subject after *in vivo* exposure to wavelengths of 360 mμ and subsequent *in vitro* incubation in tyrosine-phosphate buffer. Biopsy specimen obtained 120 hours after irradiation. Counterstained with hematoxylin and eosin. Magnification, × 268.

studied histochemically. Biopsy specimens were obtained before and at various intervals after irradiation with wavelengths of 360, 400 and 440 mμ, as has already been described. Tyrosinase activity was present in the skin of all subjects, but only after an interval of 48–72 hours in biopsy specimens from irradiated areas. In sections from control specimens (unirradiated), which had been incubated in buffered tyrosine solution, the basal cells contained very little preformed melanin, and at the epidermal-dermal junction there were no dendritic melanocytes which contained tyrosinase activity (Fig. 3). A similar distribution of melanin was seen in sections of biopsy specimens from control areas which had been incubated in simple phosphate buffer. After sections of biopsy specimens from irradiated areas had been incubated in tyrosine-phosphate buffer, on the other hand, a deposition of brown and dark brown melanin granules could be seen in the cytoplasm and the dendritic processes of the melanocytes (Figs. 4, 5). The melanocytes were darkly pigmented and showed high tyrosinase activity. The cytoplasm and the dendrites of the melanocytes from irradiated skin which had been incubated in the simple phosphate buffer also contained melanin granules, but these were very light in color. The presence of tyrosinase activity and the formation of new melanin in human skin which has been irradiated by light waves greater than 320 mμ is thus corroborated by both histochemical and visual data.

DISCUSSION

The immediate pigment-darkening (IPD) induced by light of wavelengths between 320 and 640 mμ, which has been postulated to be an oxidation reaction, can be explained theoretically in terms of the absorption of light energy by melanoprotein molecules and the transmission of this energy by conduction bands. Szent Györgi (13) has pointed out that biological proteins actually have conduction bands and that they can therefore act as semiconductors for the transmission of energy. The excitation of electrons in melanin which has absorbed light and the transmission of these electrons through intrinsic semiconductors such as melanin could bring about this oxidation. Fading or lightening of the color of immediately darkened melanin would appear then to be due to the redox system of the cells. Much additional work is needed in this field to explain this hypothesis.

Melanization is the common response of epidermal tissue to injury such as that caused by ultraviolet light with wavelengths less than 320 mμ, roentgen rays, thorium radiation and the photodynamic and photosensitizing action of synthetic dyes and furocoumarins. Following exposure to ultraviolet or roentgen rays, human dendritic melanocytes are reported (14) to enlarge, become branched and give a more pronounced dopa-oxidation reaction than do those of unirradiated skin. Fitzpatrick *et al.* (11) have also reported the activation of tyrosinase by ultraviolet radiation from a quartz, mercury-vapor lamp, and have demonstrated the presence of tyrosinase in human skin. If one examines critically all of the agents which augment pigmentation, it becomes evident that their primary effect is to injure the cells of the epidermis in a manner which produces hyperplasia of the malpighian cells and thickening of the stratum corneum. Active proliferation is manifested by the rapid increase of mitosis in the basal cells. An increase in the rate of cell division must be accompanied by increased synthesis of ribonucleic acid, protein and the particulate structures necessary for cellular activity.

The alterations of cell chemistry that follow irradiation are not adequately understood because pathways of energy dissipation remain obscure and the structure and properties of biologically important cellular molecules after irradiation are still unknown. Taking into account the amount of energy associated with light of the wavelengths studied, it would appear that the cellular changes or injuries induced by this irradiation would be most likely to affect cytoplasmic particles and cytoplasmic enzymes.

Various schemes have been proposed to explain the mechanism by which ultraviolet induces pigmentation: *e.g.*, (*a*) it brings about the oxidation of tyrosine to dopa, which in turn catalyzes the tyrosine-to-melanin reaction (11); (*b*) it causes a decrease in the number of available epidermal sulfhydryl groups which inhibit tyrosinase and thus releases active tyrosinase (15); (*c*) it injures the cells in such a way as to induce increased mitotic activity with consequent rapid turnover of epidermal cells and a resultant increase in the demand for cellular constituents (see above). The hyperplasia, active cell proliferation and thickening of the epidermis that one observes after ultraviolet irradiation indicate that

cellular injury is at the bottom of most of these changes.

Recent electron-microscope studies of the fine structure of melanocytes and biochemical studies of the subcellular particles isolated from pigmented tissues by density-gradient and ultracentrifugation have made it possible to outline consecutive stages in the development of melanin granules (16, 17). Melanin, the secretory product of specialized secretory cells (*melanocytes*) has been found to occur both in cell organelles with a distinctive internal structure, which contain tyrosinase activity and are of varying size (*melanosomes*), and in heavily pigmented particles which contain no tyrosinase activity (*melanin granules*). Tyrosinase, the polypeptide present in melanosomes, is thought to be synthesized in or on the ribonucleoprotein particles of the melanocyte and then transferred to the Golgi area where it is separated into quanta, each surrounded by its own membranous envelope (18). "Structures of this sort, *i.e.*, melanosomes, gradually become melanized within the cytoplasm by melanin, the product of the tyrosine-tyrosinase reaction. As melanin accumulates on the internal network, the external membrane gradually becomes thicker and the density of the enclosed particles increases until the interstices of the inner network have been filled in. Eventually, each graunule becomes a uniformly dense and structureless unit, the melanin granule, which is incapable of further melanin formation." It would seem natural, therefore, that in the course of repair a cellular injury produced by ultraviolet radiation would initiate increased synthesis of protein and other cellular structures. With this rapid metabolic turnover and the increased demand for cellular constituents, it would be logical for tyrosinase synthesis and the development of melanosomes to increase within the melanocyte. Such augmentation of melanin-granule formation as the result of cellular injury seems to constitute a credible explanation of the mechanism of new melanin formation. With histochemical technics, it has been possible to demonstrate the presence of newly formed, active tyrosinase in skin which has been irradiated with long-wave ultraviolet and visible light.

Until now, it has been thought that primary melanization could be induced only by the erythemal spectrum (2,900–3,200 Å). The data presented here disprove this hypothesis and show that new melanin formation *can* be induced by long-wave ultraviolet or even by visible light if a proper amount of energy is available to induce within the cell certain changes that demand repair. The special effectiveness of light of wavelengths of 254 and 297 mμ in the induction of primary melanization is due to: (*a*) the large amount of light energy associated with them; (*b*) high quantum efficiency. Quite obviously, the induction of primary melanization by long-wave ultraviolet (3,200–4,000Å) and visible (4,000–6,500 Å) light requires more energy than its induction by the erythemal spectrum (2,900–3,200 Å). To say that primary melanization can be evoked *only* by the erythemal spectrum, however, is erroneous; in addition to immediate pigment-darkening, long-wave ultraviolet and visible light can initiate primary melanization if sufficient energy is available.

SUMMARY

Until now, it has been thought that primary melanization (the formation of new melanin) could be induced *only* by light of wavelengths between 2,900 and 3,200 Å (the erythemal spectrum). The data reported here show that this upper limit is too low and that both long-wave ultraviolet (wavelengths 3,200 -4,000 Å) and visible light (wavelengths 4,000 - 6,500 Å) can also induce the formation of new melanin as well as immediate darkening of preformed melanin. The data were obtained from twenty-one subjects who were irradiated with a narrow band of monochromatic light from a high-intensity monochromator.

This investigation shows that the spectrum which induces immediate pigment-darkening does not lie within the limits previously accepted (wavelengths 3,000–4,300 Å), but extends from 3,000–6,500 Å and perhaps up to 7,000 Å. A few preliminary observations indicate that immediate pigment-darkening may be an oxidation reaction which takes place in the deeper layers of the epidermis. The authors suggest that melanoprotein molecules may act as semiconductors and that transmission by these semiconductors of the electrons of melanoprotein molecules which have been activated by aborbed light may induce immediate pigment-darkening.

Histochemical determinations show that punch-biopsy specimens taken from areas of skin which have been irradiated with long-wave ultraviolet and visible light contain newly formed, active tyrosinase and newly formed melanin

granules. This indicates that new melanin is being formed. Ultraviolet irradiation appears to cause cellular injury which initiates increased synthesis of proteins and other structures within the cell. It seems likely that augmented synthesis of melanosomes and tyrosinase and increased formation of melanin granules are integral processes in the formation of new melanin.

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DISCUSSION

DR. FARRINGTON DANIELS, Chicago, Ill.: This is certainly a fine contribution to the study of the effects of light on the skin. I suppose the reason the findings differ from previous findings is that the authors have new resources at their disposal, consisting of the monochromator and the tyrosinase system which is a conclusive and sensitive demonstration of new melanin formation. I must confess when these findings were first called to my attention, I was very skeptical but the evidence as presented is convincing.

DR. HERBERT MESCON, Boston, Mass.: Why does the melanin that is formed immediately in response to change in different wavelengths fade so rapidly? The fact that any protein in general may actually induce melanization in a half hour or twenty minutes does not hold for production of new melanin in the oxidized form.

DR. M. A. PATHAK, (in closing): The immediate pigment-darkening reaction can be explained in terms of absorption of light energy by melanoprotein molecules and the transmission of this energy by conduction bands. Recent electron spin-resonance studies of melanin by Dr. Mason of Portland, Oregon, have shown the existence of a relatively high concentration of free electrons. The concentration of free electrons was found to increase upon irradiation. It has been suggested that melanin polymer acts as a one-dimensional semi-conductor, with bound protons producing electron traps in the system. Light can induce excitation of these electrons and eventual oxidation by losing electrons.